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Effects of Chlorine Dioxide on Thyroid Function in
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The submitter of these studies was neither the sponsor of this study nor conducted it and does not know whether it has been conducted in accordance with 40 CFR Part 160.

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EFFECTS OF CHLORINE DIOXIDE ON THYROID FUNCTION IN NEONATAL RATS

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Chlorine dioxide (ClO_2), an alternative to chloramine for drinking water disinfection, has been implicated as a potential antithyroid agent (Bercz et al., 1982). Because antithyroid compounds are known to alter neurobehavioral development, the present study was designed to determine if perinatal exposure to ClO_2 affects behavioral activity in rat pups. The activity cage system was designed to monitor the development of locomotor activity of a litter of pups between ages 14-21 d. Pups were exposed to ClO_2 either directly, by gavaging 14 mg/kg from age 5 to 20 d, or indirectly via their dams' drinking water in concentrations of 2, 20, or 100 mg/l from gestation to weaning (21 d postpartum). Although the activity of the indirectly exposed group was not different from controls, the gavaged group showed significantly depressed activity for d 18 and 19 postpartum. The T_4 levels of the 21-d-old pups was significantly depressed in the 100-mg/l ClO_2 group. The gavaged pups showed an even greater T_4 depression, which correlates with their activity levels. These data support the hypothesis that ClO_2 affects thyroid function and suggests that a slight depression in T_4 can result in developmental delays.

INTRODUCTION

Thyroid hormones are known to influence growth and development of the central nervous system in mammals (Hamburgh et al., 1965; Shapiro, 1971). A deficiency of thyroid hormones during the critical periods of brain development can lead to permanent neurological and behavioral impairment (Schalock et al., 1979). Using a water escape response, Schalock et al. (1979) studied the long-term effects of induced neonatal hypothyroidism in rats and found a decreased performance on avoidance

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This manuscript has been EPA peer reviewed. Mention of trade names or products does not imply EPA endorsement.

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and escape learning. This was attributed to depressed thyroxine levels, desensitizing receptors to the catecholamines requisite in both avoidance and escape learning (Latane and Schacter, 1972).

In another study designed to monitor rat pup activity and the emergence of a home orientation behavior, Hamburgh et al. (1977) observed that these parameters were depressed in the hypothyroid pups. This test monitored the development of orientation responses to the home nest. On d 14 postpartum, 85% of the control pups had opened their eyes and oriented to the home nest. The hypothyroid pups were less active and did not display this behavior until d 18 postpartum, which corresponded with their age of eye opening.

Several studies have shown that hypothyroid neonates exhibit decreased neural vascularization, delayed myelinogenesis and synaptogenesis, and decreased body and organ weights (Oklund and Timiris, 1977; Hamburgh et al., 1977; Kikuyama et al., 1974; Balázs et al., 1969; Eayers, 1954). Altered thyroid function may be attributed to a number of factors, including genetic disorders, malfunction of the adenohypophysis, or the exposure to chemicals that affect synthesis of thyroid hormones.

Chlorine dioxide (ClO_2) has been proposed for use as an alternative to chlorine for the disinfection of drinking water. In a recent study that involved exposure to various concentrations of ClO_2 in drinking water, Bercz et al. (1982) reported that monkeys in the high-dose group (100 mg/l; 9 mg/kg·d) showed depressed thyroxine levels. Because antithyroid compounds are known to alter neurobehavioral development, the present study was designed to determine if perinatal exposure to ClO_2 affects the thyroid and behavioral activity of rat pups. Pursuit of this problem was felt to be important because current practice in drinking water disinfection could suggest that residuals of 0.5-2.0 mg ClO_2 /l could be present in drinking water.

METHODS

Sprague-Dawley rats were exposed either directly or indirectly to ClO_2 in drinking water. Litters born to the exposed and unexposed females were culled to 8 male pups at parturition. An excess number of exposed and unexposed females were bred in order to obtain a quorum of litters consisting of at least 8 male pups. At 10 d of age (postpartum), litters were placed in cages designed to measure locomotor activity. Upon weaning, pups were removed from these cages and serum samples were collected to assess thyroid function.

Direct Exposure

Pups born to unexposed dams were administered ClO_2 by oral gavage. A daily dose of 14 mg/kg was given from age 5 to 20 d postpartum. Controls received a comparable amount of distilled water. Pups were weighed weekly and age of eye opening was noted.

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Indirect Exposure

Sixty-d-old females were given ClO_2 at 2, 20, or 100 mg/l, or propylthiouracil (PTU) at 5 mg/l as a positive control, in their drinking water from 2 wk prior to mating until the pups were weaned at 21 d of age. PTU, a well-known antithyroid compound, was used strictly for comparative purposes with respect to neonatal hypothyroidism. Controls received distilled water. Dam food and water consumption as well as dam and pup body weights were monitored weekly. Age of eye opening for pups was also noted.

Behavioral Measurements

The testing system described by Crofton et al. (1980) is designed to measure locomotor activity between the ages of 10 and 21 d. The unit of measurement in this system is the activity of individual litters. Litters are housed in a cage ($31 \times 36 \times 17.5$ cm) in which the dam is restricted. Litter activity is measured when pups cross into a smaller compartment ($17 \times 27 \times 15$ cm) through small connecting holes and break the path of a photo-beam. Litter activity is monitored continuously for 10 d by an HP 9825 computer, which records the data at 10-min intervals. Data from the activity cage was analyzed by total daily activity for each day between 14 and 21 d postpartum.

Thyroid Function

Thyroid function was determined in dam and pup serum in the indirectly exposed animals and controls and from pup serum in pups receiving the direct exposures by triiodothyronine (T_3) and thyroxine (T_4) radioimmunoassays (Corning Medical and Scientific; Medfield, Mass.). Blood samples were collected from dams by cardiac puncture and from pups by decapitation. Serum, separated from the samples, was frozen prior to analysis.

Statistical Analysis

Statistical analysis of all data was conducted using Statistical Analysis System analysis of variance (SAS ANOVA) procedures.

The data for the activity cages were analyzed by total daily activity and by light and dark photoperiods separately. The mean count for each photoperiod for each day (between age 10 and 21 postpartum) as well as the mean count per hour for each day were compared by ANOVA. The litter was the unit of comparison for each measurement (i.e., body weight, eye opening, activity, and T_3 and T_4 levels).

RESULTS

In the experiment where dams were exposed to ClO_2 or PTU through their drinking water, there were no statistical differences in dam body weight. No differences were seen in the weights of pups born to ClO_2 .

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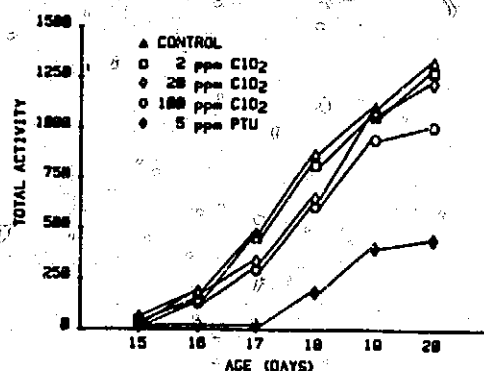


FIGURE 1. Litter activity for pups indirectly exposed through dams' drinking water. Control, $n = 13$ litters; 2 ppm ClO_2 , $n = 16$; 20 ppm ClO_2 , $n = 16$; 100 ppm ClO_2 , $n = 15$; 5 ppm PTU, $n = 10$.

exposed dams (indirect exposure regime); however, significant differences were observed between control and PTU-exposed pups. Pups indirectly exposed to PTU weighed significantly less ($p < 0.01$) than controls from 14 to 21 d of age. On d 14 the mean pup weight for the PTU pups was 19.6 g, as opposed to 30.5 g for the controls. The PTU animals weighed 21 g by d 21, while the control animals weighed an average of 49 g. Mean pup weights for the ClO_2 -gavaged pups were also significantly less ($p < 0.05$) than control animals from 14 to 21 d of age; however this depression was not as severe as in the PTU exposure. Mean weights were 20 and 24 g for ClO_2 and control pups, respectively, on d 14 postpartum. On d 21 the ClO_2 pups weighed 31 g, compared to 46 g for the controls. The age of eye opening (14–15 d postpartum) was not different between control and ClO_2 -exposed pups for either route of exposure. PTU pups, however, were significantly older (17–18 d) when they opened their eyes.

The activity levels for the pups indirectly exposed ClO_2 at 100 mg/l were consistently lower than controls; however, these differences were not significant ($p = 0.08$; Fig. 1). The degree of variability between control litters may be responsible for the nonsignificant results. Pups exposed indirectly to PTU showed significantly depressed activity levels ($p < 0.01$) throughout the experiment.

The pups directly exposed to ClO_2 showed a significant depression in total activity levels for d 18 and 19 postpartum (Fig. 2; $p < 0.05$). Total activity levels on d 18 were 500 ± 136 counts for the ClO_2 group, as compared to 740 ± 79 total counts for the controls. A similar difference continued through d 19 when the ClO_2 pups registered 750 ± 47 counts from the day while controls had slightly over 1000 ± 58 counts. By d 20 the difference between the groups was reduced to less than 100 counts.

Serum levels of thyroid hormones were affected by both direct and indirect exposure to ClO_2 . In the case of the indirect exposure to ClO_2 at 100 mg/l, T_4 levels were significantly depressed and T_3 levels were signifi-

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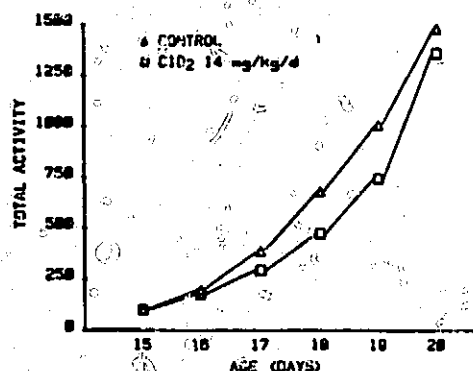


FIGURE 2. Litter activity for pups exposed directly by gavage. Control, $n = 15$; ClO₂, $n = 18$.

cantly elevated (Fig. 3). Direct exposure to ClO₂ by gavage also significantly depressed T₄ levels (Fig. 4). A much greater depression of T₄ and T₃ was observed ($p < 0.01$) in pups exposed indirectly to PTU. In no case, including PTU exposure, were serum levels of T₃ and T₄ affected in dams at the end of the experiment (d 21 postpartum).

Figure 5 illustrates the relationship between serum T₄ levels in 21-d-old pups and their activity measured in the smaller compartment of the activity cage at 20 d postpartum for all treatments. The T₄ levels are shown to be directly correlated with activity levels ($r = 0.965$).

DISCUSSION

Neonatal hypothyroidism is classically defined by depressed thyroid hormone levels, and can be further characterized by delayed eye opening and decreased body weights and activity (Schalock et al., 1979; Ham-burgh et al., 1977; Eayers, 1971; Shapiro, 1971). These characteristics were clearly evident in the PTU-exposed pups, which also exhibited other

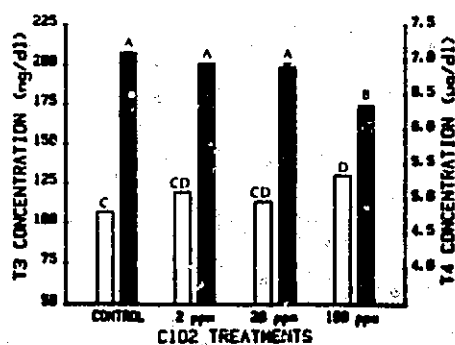


FIGURE 3. Mean serum T₃ and T₄ levels for pups indirectly exposed to ClO₂. Open bars are T₃; closed bars are T₄. Means sharing the same letters are not statistically different.

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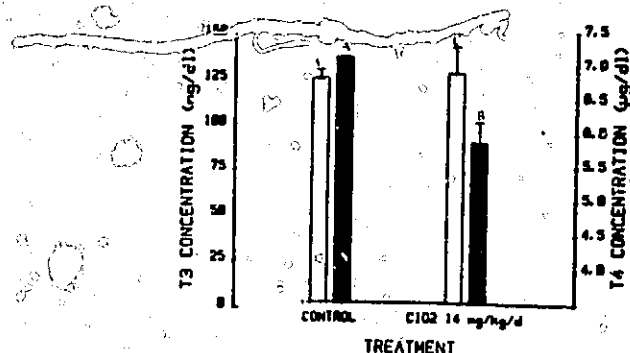


FIGURE 4. Mean serum T_3 and T_4 levels for pups gavaged with ClO_2 . Open bars are T_3 ; closed bars are T_4 . Means sharing the same letters are not significantly different.

cretinoid attributes such as decreased body size, tremors, and uncoordinated body movements (Schwark 1978).

Not all of these characteristics were exhibited by the neonatal rats exposed directly or indirectly to ClO_2 . The directly exposed group showed decreased body weights, depressed locomotor activity, and depressed T_4 levels. The group indirectly exposed to ClO_2 at 100 mg/l exhibited depressed activity (although not statistically significant) and significantly depressed T_4 levels. The significantly lower T_4 levels in these groups and altered locomotor activity levels indicate a distinct hypothyroid effect. The increased effect on the directly exposed group is more likely due to the increased dosage of ClO_2 as compared to the indirectly exposed group.

Thyroxine (T_4) deficiency inhibits the synthesis of growth hormone (Akuyama et al., 1974) and depresses protein synthesis and DNA and RNA levels in the developing rat cortex and cerebellum (Pasquini et al., 1967;

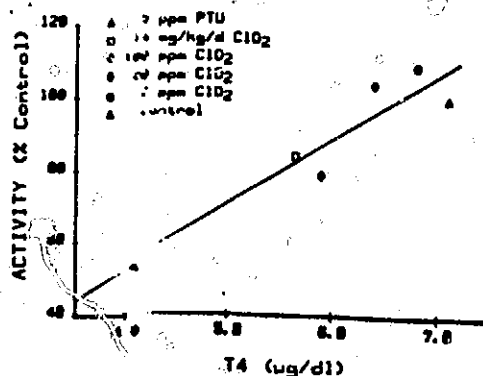


FIGURE 5. Correlation between T_4 levels (from 21-d-old pups) and total activity levels (as a percent of controls) of 21-d-old pups.

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Balázs et al., 1977). These events delay the maturation of neuronal and glial cells in the neonatal brain, involving cell proliferation, migration, and differentiation (Bass et al., 1977; Coulombe et al., 1980). The absence or insufficiency of thyroid hormones during this time can lead to irreversible damage to the developing nervous system, which can lead to behavioral impairments (Eavers, 1971). Therefore behavioral studies are a valuable means of assessing the functions of the nervous system and, in particular, the evaluation of motor skills (Balázs et al., 1977).

The differences in activity beginning at d 15 postpartum correspond with the age of eye opening (Hamburgh et al., 1977). While there were no differences between the age of eye opening for the ClO_2 exposures, the PTU group was significantly late in opening their eyes, contributing to their delayed locomotor activity development.

Results from this study show a highly significant correlation between locomotor activity and T_4 levels. This suggests that even subtle depressions of thyroid function may have significant effects on neurobehavioral development. The mechanisms by which ClO_2 affects thyroid function remain unclear. The elevated T_4 and depressed T_3 levels seen in the 100-mg/l ClO_2 group suggests an alteration in the availability of iodide. Availability of iodide for this purpose could be affected by a number of factors involved in T_3 and T_4 biosynthesis. These include the impairment of iodine absorption in the gastrointestinal tract, inability of the thyroid to concentrate iodide from the blood, impairment of the pituitary thyroid hormonal feedback mechanism, or interference with the peroxidase system needed for iodination and organification of T_3 and T_4 constituents.

It is unlikely that ClO_2 acts directly on the thyroid. Upon ingestion, ClO_2 is rapidly metabolized to ClO_2^- and ClO_3^- . However, Bercz et al. (1982) have previously shown that these metabolites do not depress T_4 levels in the monkey. So the ClO_2 -thyroid relationship remains a complicated one and may involve organic reactions of by-products of ClO_2 within the gastrointestinal tract.

It should be noted that the effects of ClO_2 and PTU on thyroid function seen in the indirectly exposed pups was produced in the absence of any measurable depression of T_3 and T_4 in the dam. Therefore, it appears that thyroid function of a neonatal rat is more sensitive to the antithyroid effects of these agents than is that of the adult.

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APPENDIX I

The active ingredient in ANTHIUM DIOXIDE, 5% stabilized chlorine dioxide is sodium chlorate. The more ClO_2 is evolved, the lower the pH.

Pups exposed indirectly to 2, 20 or 100 mg/l of ClO_2 in their drinking water showed no difference in activity compared to the controls. Gavaged rats (at 14 mg/kg.) showed depressed activity for day 18 and 19 postpartum.

Ingestion or absorption from our products in approved uses will be very low or nil compared to these dosages.

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